

The samples are placed on the convex cooling block of a Worthington migration unit which is cooled to 4° C. The buffer chambers are each filled with 500 ml of the tris-sodium barbital-barbital buffer. The voltage on the power unit is set for 200 volts, and a timer is set to run the electrophoresis for two hours and thirty minutes.

After the electrophoresis is complete, the gel is overlaid with stain consisting of 8 Phadebas amylase tablets dissolved in 10 ml of a 0.9% saline solution supplemented with 0.5% bovine serum albumin. The gel is maintained in a moist chamber at 56° C. for 2½ hr., and then the gel is rinsed in distilled water and soaked in distilled water for 5 minutes. The gel is then fixed in reagent grade ethanol for 2 hours. The fixed gels are rinsed briefly in distilled water.

To dry the gel, a sheet of blotter paper is applied over its surface. A glass plate is placed over the blotter paper, and a 1 kg weight is placed on the plate and left on the plate for 10 min. The procedure is repeated with a fresh sheet of blotter paper. Drying is completed by placing the blotted gel in a vented oven at 50° C. for 15 minutes.

To quantitate the bands, the gels are scanned using a Beckman CDS-200 densitometer set at a transmission mode to read at 600 nm. The individual peaks are calculated as a percentage of the total.

The FIGURE is a photograph of isoamylase electrophoresis gel prepared according to the above Example. It can be seen from the FIGURE that the P<sub>1</sub>, P<sub>1b</sub>, P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>, S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> bands are clearly separated.

Although the test has been described above in terms of a protocol in which the gels and the electrophoresis reagents are freshly prepared, the gels may be prepared and packaged in sealed pouches and the running buffer packaged as dry mixes of the component reagents. As such, the electrophoresis supplies may be sold as a kit to clinical laboratories where testing of samples can be conducted with very little advance preparation by the clinical technician.

The adaptability of preparation as a prepacked test and the clear separation of the isoamylase bands are major advantages of the invention. In particular, the clear separation of all clinically significant isoamylase bands by the method of the present invention is an important improvement over previously described electrophoresis techniques.

While the invention has been described in terms of a certain preferred embodiment, modifications obvious to one with ordinary skill in the art may be made without departing from the scope of the present invention.

Various feature of the invention are set forth in the following claims.

We claim:

1. An electrophoretic method of detecting human isoamylases comprising

providing an aqueous buffer having a pH of between about 8.4 and about 9.2, said buffer consisting essentially of water, barbital anion at a concentration of between about 0.04 M and about 0.08 M, tris cation at a concentration of between about 0.03 M and about 0.07 M, sodium cation at a concentration of between about 0.03 M and about 0.07 M, said tris cation and said sodium cation being present in molar ratios of between about 0.5:1 and about 2:1,

incorporating said buffer in an agarose gel of between about 0.4 and about 1.5 weight percent agarose, applying biological samples to said gel, subjecting said gel to a direct current electrical potential of between about 50 and about 400 volts and

placing said gel in heat exchange relationship with a cooling block maintained at a temperature of between about 2° C. and about 25° C. so as not to denature the isoamylases or destabilize the gel, and developing said gel to reveal isoamylase bands, said method providing a clear separation of P<sub>1</sub>, P<sub>1b</sub>, P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>, S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> bands.

2. A method according to claim 1 including measuring the relative intensities of said developed isoamylase bands.

3. A method according to claim 1 wherein said developed isoamylase bands are measured by drying the gel and subsequently measuring the optical transmission of the gel in the region of the bands.

4. A method according to claim 1 wherein the pH of said buffer is between about 8.7 and about 8.9.

5. A method according to claim 1 wherein said gel is less than about 1 mm thick and each of said biological samples is applied in amounts providing amylase at levels of between about  $5 \times 10^{-4}$  and about  $4 \times 10^{-2}$  IU per cm of band length.

6. A method according to claim 1, said buffer containing calcium cation up to a concentration of about 0.002 M.

7. A method according to claim 1, said buffer containing lactate anion up to a concentration of about 0.002 M.

8. An electrophoresis plate for separating human isoamylases comprising a backing sheet and a gel on one surface of said sheet, said gel including an aqueous buffer with a pH of between about 8.4 and about 9.2, and consisting essentially of water, barbital anion at a concentration of between about 0.04 M and about 0.08 M, tris cation at a concentration of between about 0.03 M and about 0.07 M, sodium cation at a concentration of between about 0.03 M and about 0.07 M, said tris cation and said sodium cation being present in molar ratios of between about 0.5:1 and about 2:1, and agarose in amounts of between about 0.4 and about 1.5 weight percent of said gel.

9. A plate according to claim 8, said buffer containing calcium cation up to a concentration of about 0.002 M.

10. A plate according to claim 8, said buffer containing lactate anion up to a concentration of about 0.002 M.

11. A plate according to claim 8, wherein the pH of said buffer is between about 8.7 and about 8.9.

12. A prepackaged kit for effecting electrophoretic separation of human isoamylases comprising

a dry mixture of tris barbital, barbital and sodium barbital which when added to a given volume of water provides an aqueous buffer with a pH of between about 8.4 and about 9.2, and said buffer consisting essentially of water, barbital anion at a concentration of between about 0.03 M and about 0.08 M, tris cation at a concentration of between about 0.03 M and about 0.07 M, sodium cation at a concentration of between about 0.03 M and about 0.07 M, said tris cation and said sodium cation being present in molar ratios of between about 0.5:1 and about 2:1, and

a prepared agarose gel consisting of agarose at a concentration of between about 0.4 and about 1.5 weight percent and buffer of like composition to said aqueous buffer.

13. A kit according to claim 12 wherein said dry mixture includes calcium lactate so that said aqueous buffer contains calcium lactate up to a concentration of about 0.002 M.

14. A kit according to claim 12 wherein the pH of said aqueous buffer is between about 8.7 and about 8.9.

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